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Liang Wu

Iowa State University

Regina S. McGrane

Iowa State University

Gwyn A. Beattie

Iowa State University, gbeattie@iastate.edu

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Abstract

The biological and regulatory roles of photosensory proteins are poorly understood for nonphotosynthetic bacteria. The foliar bacterial pathogen *Pseudomonas syringae* has three photosensory protein-encoding genes that are predicted to encode the blue-light-sensing LOV (light, oxygen, or voltage) histidine kinase (LOV-HK) and two red/far-red-light-sensing bacteriophytochromes, BphP1 and BphP2. We provide evidence that LOV-HK and BphP1 form an integrated network that regulates swarming motility in response to multiple light wavelengths. The swarming motility of *P. syringae* B728a deletion mutants indicated that LOV-HK positively regulates swarming motility in response to blue light and BphP1 negatively regulates swarming motility in response to red and far-red light. BphP2 does not detectably regulate swarming motility. The histidine kinase activity of each LOV-HK and BphP1 is required for this regulation based on the loss of complementation upon mutation of residues key to their kinase activity. Surprisingly, mutants lacking both *lov* and *bphP1* were similar in motility to a *bphP1* single mutant in blue light, indicating that the loss of *bphP1* is epistatic to the loss of *lov* and also that BphP1 unexpectedly responds to blue light. Moreover, whereas expression of *bphP1* did not alter motility under blue light in a *bphP1* mutant, it reduced motility in a mutant lacking *lov* and *bphP1*, demonstrating that LOV-HK positively regulates motility by suppressing negative regulation by BphP1. These results are the first to show cross talk between the LOV protein and phytochrome signaling pathways in bacteria, and the similarity of this regulatory network to that of photoreceptors in plants suggests a possible common ancestry.

Disciplines

Agricultural Science | Agriculture | Plant Breeding and Genetics | Plant Pathology

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RESEARCH ARTICLE

Light Regulation of Swarming Motility in *Pseudomonas syringae* Integrates Signaling Pathways Mediated by a Bacteriophytochrome and a LOV Protein

Liang Wu, Regina S. McGrane, Gwyn A. Beattie

Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, USA

ABSTRACT The biological and regulatory roles of photosensory proteins are poorly understood for nonphotosynthetic bacteria. The foliar bacterial pathogen *Pseudomonas syringae* has three photosensory protein-encoding genes that are predicted to encode the blue-light-sensing LOV (light, oxygen, or voltage) histidine kinase (LOV-HK) and two red/far-red-light-sensing bacteriophytochromes, BphP1 and BphP2. We provide evidence that LOV-HK and BphP1 form an integrated network that regulates swarming motility in response to multiple light wavelengths. The swarming motility of *P. syringae* B728a deletion mutants indicated that LOV-HK positively regulates swarming motility in response to blue light and BphP1 negatively regulates swarming motility in response to red and far-red light. BphP2 does not detectably regulate swarming motility. The histidine kinase activity of each LOV-HK and BphP1 is required for this regulation based on the loss of complementation upon mutation of residues key to their kinase activity. Surprisingly, mutants lacking both *lov* and *bphP1* were similar in motility to a *bphP1* single mutant in blue light, indicating that the loss of *bphP1* is epistatic to the loss of *lov* and also that BphP1 unexpectedly responds to blue light. Moreover, whereas expression of *bphP1* did not alter motility under blue light in a *bphP1* mutant, it reduced motility in a mutant lacking *lov* and *bphP1*, demonstrating that LOV-HK positively regulates motility by suppressing negative regulation by BphP1. These results are the first to show cross talk between the LOV protein and phytochrome signaling pathways in bacteria, and the similarity of this regulatory network to that of photoreceptors in plants suggests a possible common ancestry.

IMPORTANCE Photosensory proteins enable organisms to perceive and respond to light. The biological and ecological roles of these proteins in nonphotosynthetic bacteria are largely unknown. This study discovered that a blue-light-sensing LOV (light, oxygen, or voltage) protein and a red/far-red-light-sensing bacteriophytochrome both regulate swarming motility in the foliar pathogen *Pseudomonas syringae*. These proteins form an integrated signaling network in which the bacteriophytochrome represses swarming motility in response to red, far-red, and blue light, and LOV positively regulates swarming motility by suppressing bacteriophytochrome-mediated blue-light signaling. This is the first example of cross talk between LOV and phytochrome signaling pathways in bacteria, which shows unexpected similarity to photoreceptor signaling in plants.

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Address correspondence to Gwyn A. Beattie, gbeattie@iastate.edu.

Light is a dominant environmental component of many terrestrial and aquatic habitats. Prokaryotes occupy microhabitats that differ in the quantity and quality of light exposure; therefore, their photosensory systems may be optimized for sensing or capturing light in the spectra available to them, as illustrated by the various pigments in distinct phototrophic species (1). Photosynthetic and nonphotosynthetic prokaryotes have photosensory proteins that enable the integration of light into environmental sensing networks. Prokaryotic photosensory protein families include the red/far-red-light-absorbing bacteriophytochromes, the green- or blue-light-absorbing rhodopsins, photoactive yellow proteins, the blue-light-absorbing photolyases/cryptochromes, and BLUF (blue light using flavin adenine dinucleotide [FAD]) and LOV (light, oxygen, or voltage) domain-containing proteins (2–5). The presence of multiple photosensory proteins in a single cell should thus enable an integrated response to the full light

spectrum. In terrestrial habitats, for example, organisms at different depths in a soil column will encounter distinct ratios of red and far-red light, with those near the soil surface or on a plant surface also encountering blue light, the intensity of which will be mediated by depth and overhead vegetation. Although multiple photosensory proteins may be integrated into a single signal transduction network, as was shown recently for two blue-light responsive proteins regulating photosynthesis genes in *Rhodospirillum rubrum* (6), prokaryotic photosensory proteins that respond to diverse spectra have not yet been shown to be integrated into a single coordinated network.

The species *Pseudomonas syringae* is found in several habitats that are dominated by light, including leaves where it can function as a foliar pathogen, surface soils, mountain creeks and lakes, and snowpack (7). The genome sequences of several strains of this species indicate the presence of multiple photosensory proteins.

For example, the bean pathogen *P. syringae* pv. *syringae* strain B728a encodes two bacteriophytochromes, BphP1 and BphP2, and a LOV domain-containing histidine kinase, LOV-HK (5, 8). Although a role of light in the life cycle of this pathogen has not been investigated, we envision that light could provide cues for movement and relocation, survival, and even virulence based on its coordination of bacterial physiology with the plant's photo-cycle. In this work, we identified roles for the bacteriophytochrome and LOV-HK proteins in regulating swarming motility in *P. syringae* and investigated whether the proteins form an integrated light-responsive network.

The most abundant photoreceptors in bacteria are phytochromes (9). These proteins covalently bind a bilin chromophore, thus enabling the detection of red or far-red light through the isomerization of a double bond in the bilin (8). Phytochromes have been found in 17% of all sequenced bacterial genomes (9). The phytochromes of plants and cyanobacteria generally bind phytychromobilin and phytycyanobilin, respectively (10, 11), whereas bacteriophytochromes bind biliverdin (8). The bacteriophytochromes known thus far exhibit an exclusively red/far-red photochromism and exhibit reversible photoconversion between two stable conformations, a red-light-absorbing Pr form and a far-red-light-absorbing Pfr form (8). The vast majority of bacteriophytochromes, including *P. syringae* BphP1 and BphP2, have a C-terminal histidine kinase domain fused to their N-terminal biliverdin-binding domain (8). Studies with BphP1 from *P. syringae* pv. tomato strain DC3000 found that biliverdin binding is required for the red/far-red-light-driven autophosphorylation of BphP1 and that the Pfr form showed significantly higher autophosphorylation activity than the Pr form (8). Biochemical studies of BphP2 have thus far been limited by its resistance to purification (12).

The downstream physiological outputs from bacteriophytochromes are known for surprisingly few bacteria. Among the anoxygenic photosynthetic bacteria, bacteriophytochromes have been found to help activate a switch between photosynthetic and respiratory metabolism (13) and to regulate the production of photosynthetic systems (14) and the synthesis of light-harvesting systems to optimize light collection (15) have been found. The cyanobacterial phytochromes Cph1 and Cph2 regulate the growth rate under far-red light and red light, respectively (16), with Cph2 also inhibiting phototaxis toward blue light, suggesting a blue-light-responsive function (17, 18). Cyanobacteria also have phytychrome-like cyanobacteriochromes that regulate pigment production (19) and phototaxis (20), but these cyanobacteriochromes are distinct from phytochromes in their domain structure, their use of phycoviolobilin as a chromophore, and their red/green or blue/green photochromism (21). Among the non-photosynthetic bacteria, a *Deinococcus radiodurans* bacteriophytochrome regulates the synthesis of a pigment that is thought to protect cells from light-induced cellular damage (22), whereas a bacteriophytochrome in *Pseudomonas aeruginosa* was experimentally associated with heat shock (23). As with *P. syringae*, a biological role has not yet been identified for the bacteriophytochromes of *Agrobacterium tumefaciens* (24, 25).

Photosensory proteins containing a LOV domain, designated LOV proteins, are widespread in plants and prokaryotes although absent in animals (26, 27). These proteins enable a response to blue light due to conformational changes that result from light-mediated protein-flavin adduct formation at a conserved cysteine

in the LOV domain (5), which can subsequently activate a variety of C-terminal effector domains (28). About half of the prokaryotic LOV proteins are LOV histidine kinases (LOV-HKs) (29). Characterization of LOV-HK of *P. syringae* pv. tomato strain DC3000 showed that it associates primarily with a flavin mononucleotide (FMN) and secondarily with FAD (30) and exhibits blue-light-mediated autophosphorylation (26, 30). Moreover, this protein has not only an N-terminal LOV domain followed by a histidine kinase domain but also a C-terminal response regulator (RR) domain (26, 29, 30). Following autophosphorylation, the *P. syringae* LOV-HK protein rapidly transferred the phosphate to this RR domain, at least when the domains were cloned separately (30). This domain structure is found primarily in bacterial plant pathogens (31).

Our biochemical understanding of the LOV-HK proteins contrasts sharply with our dearth of knowledge of their downstream physiological roles. These roles have been identified for only four prokaryotic LOV-HK proteins thus far. The LOV-HK protein in the foliar pathogen *Xanthomonas axonopodis* pv. *citri* contributes to light-mediated regulation of adhesion to leaves and virulence (31); this LOV-HK has the same domain structure as *P. syringae* LOV-HK. In contrast, LOV-HK proteins that have a different domain structure regulate blue-light-dependent cellular proliferation in macrophages for the animal pathogen *Brucella abortus* (5), increase intercellular attachment in the aquatic bacterium *Caulobacter crescentus* (32), and reduce attachment to abiotic surfaces and regulate exopolysaccharide production and nodulation in *Rhizobium leguminosarum* (33).

In this study, we discovered that the *P. syringae* B728a photosensory proteins BphP1 and LOV-HK regulate swarming motility, a coordinated movement of bacterial cells across a semisolid surface (34) that requires the synthesis of flagella and, in *P. syringae*, involves the secretion of a biosurfactant (35). We discovered the unusual involvement of the bacteriophytochrome BphP1 in responding to blue light as well as red and far-red light and the integration of BphP1 and LOV-HK into a network in which LOV-HK suppresses BphP1-mediated blue-light signaling. Thus, we provide the first evidence of an integrated regulatory network of phytychrome and LOV photosensory proteins in bacteria.

RESULTS

Light decreases the swarming motility of *P. syringae* B728a. An initial screen for B728a phenotypes that were impacted by light identified swarming motility as a light-regulated phenotype. When replicate aliquots of B728a cells were placed on a solid medium and were or were not exposed to white light, the cells exposed to light showed much less spreading than those in the dark during a 10- to 15-h incubation (Fig. 1A; see also Fig. S1A in the supplemental material). For organisms that spread with even colony margins, swarming motility is often quantified by measuring the colony diameter (36). To quantify swarming motility with *P. syringae*, which produces tendrils, we measured the lateral colony surface area in digital images using the Adobe Photoshop software program (Fig. 1C), similar to the approach taken in previous studies (37, 38), and found that the cells in the light spread less ($P < 0.01$) than those in the dark. Differences were not observed when both halves of the plates were kept in the dark (Fig. 1B and D). The cells in the light eventually exhibited swarming motility (see Fig. S1A in the supplemental material), which shows that light decreases swarming motility but does not repress it fully.

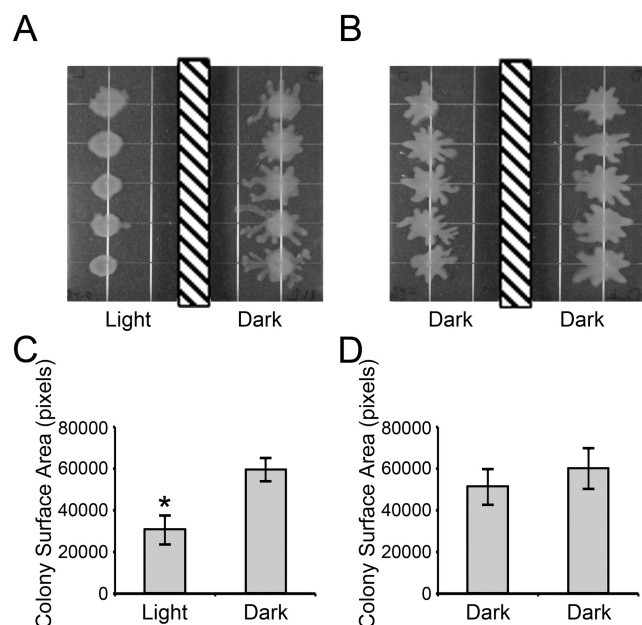


FIG 1 Light decreases the swarming motility of *P. syringae* B728a. Five replicate drops of B728a cells were inoculated onto motility plates (0.4% King's B agar) in rows that were separated by an opaque divider (indicated by cross-hatching in panels A and B). Plates were either half-covered (A and C) or fully covered (B and D) with felt and incubated under white light for 10 to 15 h. Photos were taken upon initiation of movement by colonies in the light, and the lateral colony surface area at this time was quantified, with mean values \pm SD ($n = 5$). The asterisk indicates a difference between the light and dark treatment ($P < 0.01$). Similar results were observed in three independent experiments.

Because B728a initiates tendril formation only after a period of growth under both light and dark conditions and exhibits continuous spreading thereafter, swarming motility measurements through this study were compared only among colonies within a plate and at a time before the colonies grew into each other. Strain comparisons were made based on measurements of five replicate colonies of each strain within the same plate, with measurements across plates not comparable.

The number of cells in the colonies after incubation did not differ in the light versus the dark (see Fig. S1B), which was expected given the short time (2 to 5 h) between the visible initiation of swarming motility and the colony surface area measurements. Thus, the colony surface area primarily or exclusively reflects motility with little or no contribution from growth. Similarly, although the temperature at the surface of the medium was up to 0.2°C higher on the side of the plate exposed to the light, a temperature difference of 0.4°C in the same temperature range in the dark did not alter motility (see Fig. S1C and D). Collectively, these data support that light was the primary environmental factor reducing the swarming motility in our experiments.

LOV-HK positively regulates swarming motility in response to blue light. To evaluate the role of LOV-HK in the regulation of swarming motility, we constructed a Δlov deletion mutant (see Fig. S2A in the supplemental material) and found that this mutant exhibited decreased swarming motility under both white light and blue light ($P < 0.01$) (Fig. 2A to C) but not under red plus far-red light or in the dark (Fig. 2A, D, and E). These results suggest that LOV-HK works as a positive regulator of swarming motility in

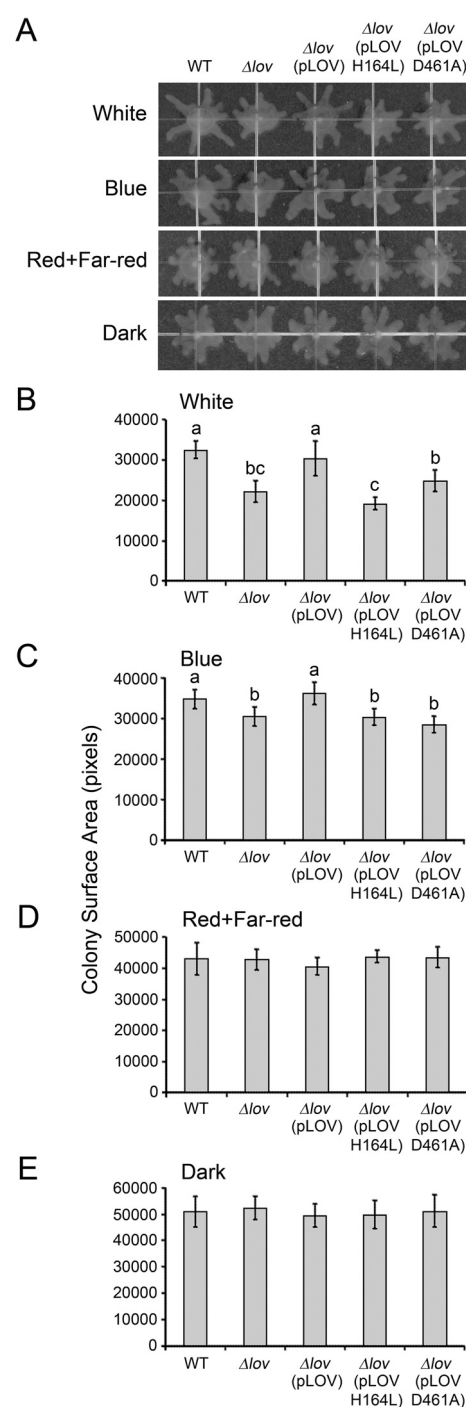


FIG 2 Contribution of LOV-HK to swarming motility under various light conditions. Motility assays were performed with wild-type B728a (WT) and several *lov* gene deletion derivatives, including a Δlov mutant, a Δlov (pLOV) complementation strain, which expresses *lov* in *trans*, and two derivatives expressing *lov* with the point mutation H164L or D461A. Five replicates of each of the strains were inoculated in a randomized order in five parallel rows on the same plate. (A) Photographs of representative colonies that developed under various light conditions. (B to E) The mean colony surface area \pm SD ($n = 5$) after 10 to 15 h under white light (B), blue light (C), red plus far-red light (red + far-red) (D), or in the dark (E). Within each panel, values indicated by different letters differ significantly ($P < 0.01$). The absence of letters indicates the absence of significant differences among the strains. Independent experiments were repeated at least three times with similar results.

response to blue light. We cloned the *lov* gene into pME6041 (39) under the control of its native promoter and then introduced the resulting construct, pLOV, into the Δlov mutant and verified *lov* expression (see Fig. S2B). Motility assays confirmed a positive regulatory role of LOV-HK based on the similarity of the motility of the Δlov (pLOV) complementation strain to that of the wild type under white and blue light (Fig. 2B and C).

LOV-HK is a histidine kinase; however, it contains an additional C-terminal response regulator (RR) domain. To study the association between phosphorylation activity and LOV-HK activation of swarming motility, we made point mutations in pLOV that altered conserved residues in the kinase domain (His-164) and in the RR domain (Asp-461). Motility assays with the Δlov (pLOV_H164L) and Δlov (pLOV_D461A) strains showed that both mutations eliminated the ability of pLOV to rescue the phenotype of Δlov (Fig. 2B and C). Although both the Δlov (pLOV_H164L) and Δlov (pLOV_D461A) strains were similar to the Δlov mutant in their motility, the Δlov (pLOV_D461A) strain showed significantly greater motility than the Δlov (pLOV_H164L) strain and significantly lower motility than the wild type and the Δlov (pLOV) strain under white light ($P < 0.01$) (Fig. 2B). Collectively, these results demonstrate that both His-164 and Asp-461 contribute to the phosphorylation activities required for full LOV-HK function and indicate that, in addition to the RR domain of LOV-HK, one or more additional RRs likely function downstream of LOV-HK signaling.

BphP1 negatively regulates swarming motility in response to red and far-red light. To identify if BphP1 is involved in light-mediated regulation of swarming motility, we deleted *bphP1* (see Fig. S2A in the supplemental material), which encodes a red/far-red-light-sensing bacteriophytochrome, and performed motility assays under white light, blue light, red plus far-red light, and in the dark (Fig. 3A). Our data show that the $\Delta bphP1$ mutant exhibited increased motility ($P < 0.01$) under white light and red plus far-red light compared to the wild type (Fig. 3B and D) but did not differ significantly from the wild type under blue light and in the dark (Fig. 3C and E). These results suggest that BphP1 acts to repress swarming motility in response to red/far-red light. To further evaluate this repression, we cloned *bphP1* into the pME6031 vector (39) under the control of the *bphO* promoter to make the complementation construct, pBphP1. The *bphO* promoter was used because *bphO* is upstream of *bphP1* in a two-gene operon. We introduced pBphP1 into the $\Delta bphP1$ mutant and confirmed that *bphP1* was expressed (see Fig. S2C). As expected, the introduction of pBphP1 reduced the motility of the $\Delta bphP1$ (pBphP1) strain to wild-type levels in white and red plus far-red light (Fig. 3B and D) but did not alter its motility under the other light conditions (Fig. 3C and E), supporting the role of BphP1 as a red/far-red-light receptor that represses swarming motility.

BphP1 is a histidine kinase, and its kinase activity requires autophosphorylation at a conserved histidine residue in the kinase domain (8). To evaluate if the kinase activity is required for repression, we made a site-directed mutation in pBphP1 that replaced the codon encoding the conserved His-530 with one encoding leucine. Motility assays with $\Delta bphP1$ (pBphP1_H530L) showed that the mutation eliminated the ability of pBphP1 to rescue the phenotype of the $\Delta bphP1$ mutant (Fig. 3B and D), indicating that the conserved His-530 is essential for the regulatory function of BphP1.

BphP1 has two photoconvertible forms, a red-light-absorbing

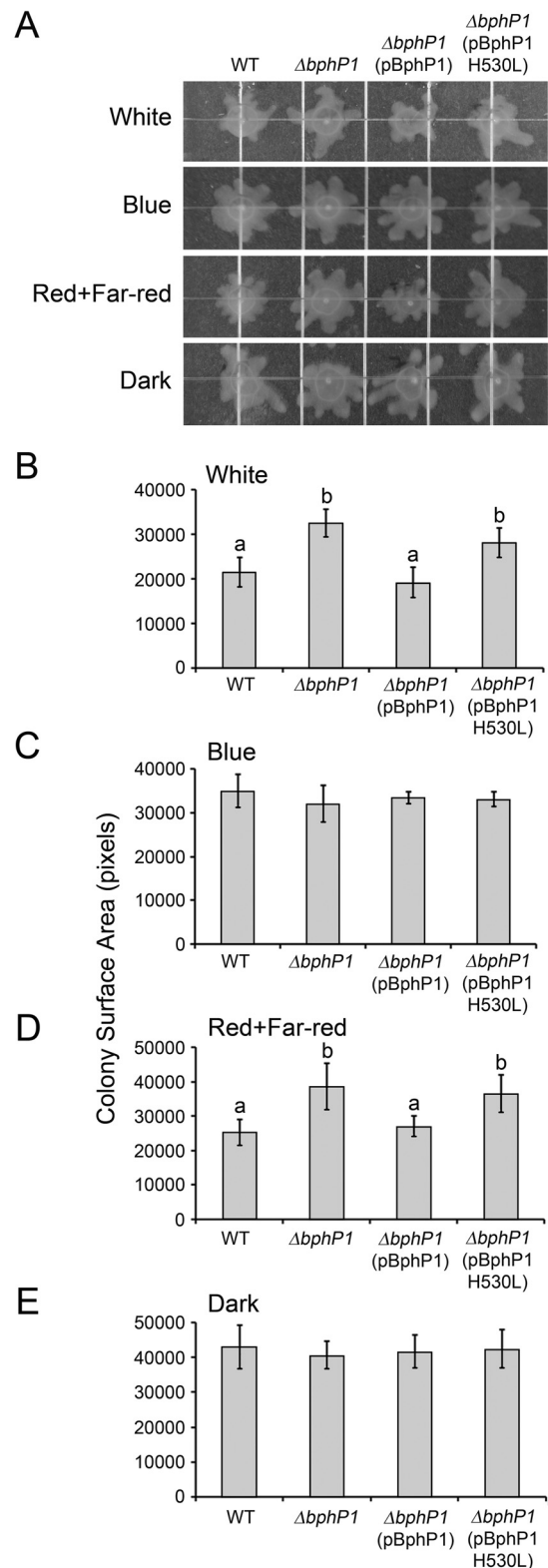


FIG 3 Contribution of BphP1 to swarming motility under various light conditions. Motility assays were performed with B728a (WT) and the *bphP1* deletion derivatives $\Delta bphP1$, $\Delta bphP1$ (pBphP1), which expresses the *bphP1* gene in *trans*, and $\Delta bphP1$ (pBphP1_H530L), which expresses *bphP1* with the point mutation H530L. The figures shown are as described for Fig. 2.

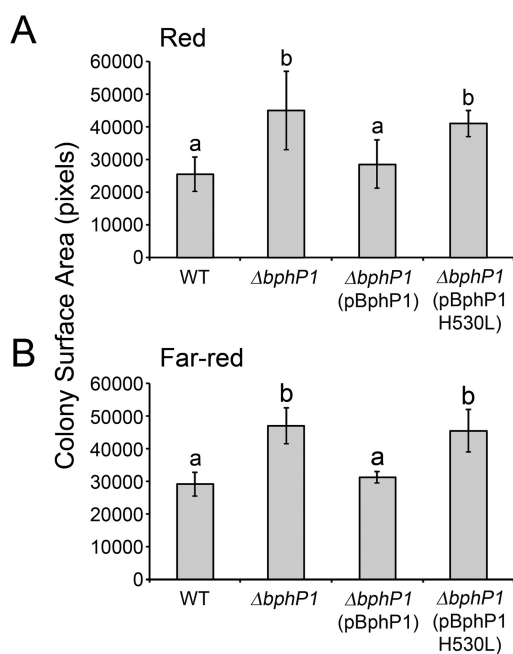


FIG 4 Contribution of BphP1 to swarming motility under either red-light or far-red-light conditions. Motility assays were performed with B728a (WT), $\Delta bphP1$, $\Delta bphP1(pBphP1)$, and $\Delta bphP1(pBphP1_H530L)$ separately under red light (A) or far-red light (B). Values are as described for Fig. 2. Independent experiments were repeated three times with similar results.

Pr form and a far-red-light-absorbing Pfr form. To further determine which form is responsible for the function of BphP1 in regulating swarming motility, we performed motility assays under either red or far-red light. Our results demonstrated that BphP1 suppressed swarming motility in response to each of these wavelengths (Fig. 4). Moreover, the loss of complementation by inactivation of His-530 (Fig. 4) indicated that the histidine kinase activity was required for BphP1 function at both wavelengths. These results suggest that BphP1 is biologically active as both Pr and Pfr forms.

A *bphP1* deletion is epistatic to a *lov* deletion in its effect on swarming motility, whereas a *bphP2* deletion has no detectable effect on swarming motility. The fact that both LOV-HK and BphP1 regulate swarming motility suggests integrated signal transduction pathways. We did not find a role for BphP2 in these pathways based on the similarity of a *bphP2* deletion mutant to the wild type in swarming motility under all of the light conditions tested (Fig. 5). A phylogenetic analysis of 18 phytochromes showed that BphP1 and BphP2 exhibited far less evolutionary divergence in *P. syringae* than in the other two species containing multiple BphP homologs, *Agrobacterium tumefaciens* and *Pseudomonas putida* (see Fig. S3 in the supplemental material). This low level of divergence suggests possible functional redundancy between these two homologs in *P. syringae*, despite the 0.8-kb difference in size of the *bphP1* and *bphP2* genes. To investigate this possibility and potential interactions among the three photosensory proteins in regulating swarming motility, we constructed a $\Delta bphP1 \Delta bphP2$ double mutant and a $\Delta bphP1 \Delta bphP2 \Delta lov$ triple mutant (see Fig. S2A). The motility of the $\Delta bphP1 \Delta bphP2$ mutant was similar to that of the $\Delta bphP1$ mutant under all of the light conditions (Fig. 5), indicating that BphP2 is not functionally redundant with BphP1 in regulating swarming motility.

The $\Delta bphP1 \Delta bphP2 \Delta lov$ mutant exhibited motility similar to that of the $\Delta bphP1$ mutant under white and blue light despite the lower motility of the Δlov mutant (Fig. 5B and C). Thus, the loss of *bphP1* is phenotypically dominant over the loss of *lov*; moreover, BphP1 is responsive to blue light in repressing swarming motility. Under red plus far-red light, the mutants lacking *bphP1* exhibited increased motility ($P < 0.01$), whereas strains with an intact *bphP1* gene, namely the $\Delta bphP2$ and Δlov mutants, showed motility similar to that of the wild type (Fig. 5D). These results indicate that only BphP1 actively regulates swarming motility in response to red and far-red light. All of the strains showed similar motility in the dark (Fig. 5E). Mutants that were deficient in the *bphO-bphP1* operon, *bphOP1*, and the *bphP2-bphR* operon, *bphP2R*, behaved similarly to the $\Delta bphP1$ and $\Delta bphP2$ mutants, respectively, and the multiple deletion mutants lacking these operons behaved similarly to the corresponding gene deletion mutants (see Fig. S4 in the supplemental material). Taken together, these results demonstrate that, in addition to its response to red and far-red light, BphP1 is involved in the response of B728a to blue light; furthermore, it functions downstream of LOV-HK in the blue-light signal transduction pathway.

LOV-HK suppresses the BphP1-mediated blue-light signaling pathway. The introduction of pBphP1 into the $\Delta bphP1 \Delta lov$ double mutant reduced the motility of the $\Delta bphP1 \Delta lov$ mutant to the level of the Δlov mutant in blue light (Fig. 6), confirming that BphP1 functions to repress swarming motility in response to blue light. Moreover, the lack of a difference in motility between the $\Delta bphP1$ and $\Delta bphP1(pBphP1)$ strains under blue light (Fig. 3A and C), coupled with evidence for blue-light activation of BphP1-mediated repression of swarming motility when the *lov* gene was absent (Fig. 6), suggests that LOV-HK acts as a repressor of the BphP1-mediated blue-light response. Moreover, whereas the motility of the wild type was not influenced by the loss of BphP1 under blue light (Fig. 6), the motility of the wild type was influenced by the loss of BphP1 under white light (Fig. 5), suggesting that BphP1-mediated repression of swarming motility was not completely silenced by the presence of LOV-HK under white light as it was under blue light. Taken together, these findings support that BphP1-mediated red/far-red-light repression of swarming motility is distinct from its blue-light signaling, with only the latter subject to LOV-HK suppression.

DISCUSSION

Our data demonstrate that nonphotosynthetic bacteria, like plants and photosynthetic bacteria, have signaling networks that integrate distinct light signals to control target behaviors. Collectively, our results support a network in which swarming motility is regulated by BphP1 and LOV-HK in the phytopathogen *P. syringae* B728a (Fig. 7). This network employs a single phytochrome responding to distinct light ranges, blue, red, and far-red light, and also a cross talk between signaling pathways mediated by a LOV protein and a phytochrome in response to a single light signal, blue light. In this network, BphP1 negatively regulates swarming motility, whereas LOV-HK positively regulates swarming motility by means of suppressing the negative regulation by BphP1 in response to blue light. Our identification of a highly quantitative phenotype, swarming motility, as a behavior regulated by light enabled us to characterize this signaling network. Thus, this network is supported by the results of motility assays performed un-

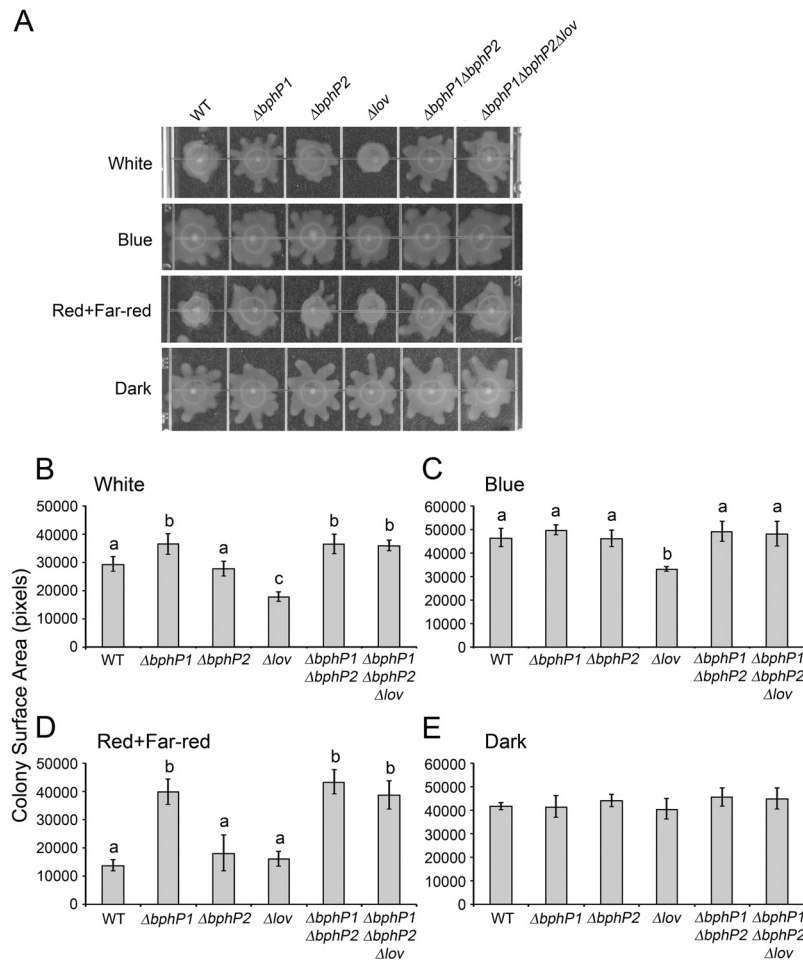


FIG 5 Impact of the loss of multiple photosensory proteins on swarming motility. Motility assays were performed with B728a (WT) and the $\Delta bphP1$, $\Delta bphP2$, Δlov , $\Delta bphP1 \Delta bphP2$, and $\Delta bphP1 \Delta bphP2 \Delta lov$ mutants, and the figures shown are as described for Fig. 2.

der multiple light ranges with various genetic derivatives of *P. syringae* B728a that were altered in the presence and expression of its three known photosensory genes, with one of these, *bphP2*, remaining phenotypically silent with regard to swarming motility.

The function of BphP1 as a negative regulator was demonstrated by the increased motility of a $\Delta bphP1$ mutant. This evidence was further strengthened by the behavior of a complementation strain that expressed the *bphOP1* operon rather than simply the *bphP1* gene at a higher level (see Fig. S5 in the supplemental material). BphO is a heme oxygenase that synthesizes biliverdin from heme as well as stabilizing BphP1 during its biosynthesis (8, 12). When the *bphOP1* operon was expressed under the control of tandem *nptII* (40) and *bphO* promoters, both *bphO* and *bphP1* genes were expressed at a much higher level than in the wild type (5.7- and 7-fold, respectively) (see Fig. S5A). Moreover, due to the stability provided to BphP1 by BphO during BphP1 synthesis (8, 12), this strain likely supported a higher level of BphP1 activity than did the $\Delta bphP1$ (pBphP1) complementation strain, which expressed the *bphP1* gene at a level that was about 3-fold higher than that in the wild type (see Fig. S2C). Overexpression of *bphOP1* magnified the suppressive effect of BphP1 in white light, in red plus far-red light, and even in blue light (see Fig. S5B) compared to the expression of *bphP1* alone (Fig. 3), consistent

with the role of BphP1 as a negative regulator of swarming motility.

We initially revealed the impact of light on *P. syringae* swarming motility based on differences in the movement of wild-type B728a cells under white light versus that in the dark. The signaling network involving BphP1 and LOV-HK identified in this study predicts that B728a should exhibit lower swarming motility under red plus far-red light than in the dark, and this prediction was confirmed in motility assays (see Fig. S6A in the supplemental material). In contrast, the interaction between LOV-HK and BphP1 signaling predicts that the swarming motility of B728a should not be suppressed under blue light, because LOV-HK suppresses negative regulation by BphP1, which is supported by the similar motility of the $\Delta bphP1$ and $\Delta bphP1$ (pBphP1) strains observed under blue light (Fig. 3A and C). However, B728a surprisingly exhibited significantly less swarming motility in blue light than in the dark (see Fig. S6B). Since LOV-HK functions as a positive regulator of swarming motility (Fig. 2) and does so by relieving the BphP1-mediated suppression of swarming motility (Fig. 5 and 6), the blue-light-mediated suppression of swarming motility shown in Fig. S6B suggests the presence of one or more blue-light-responsive regulators in addition to BphP1 that function to suppress swarming motility (Fig. 7).

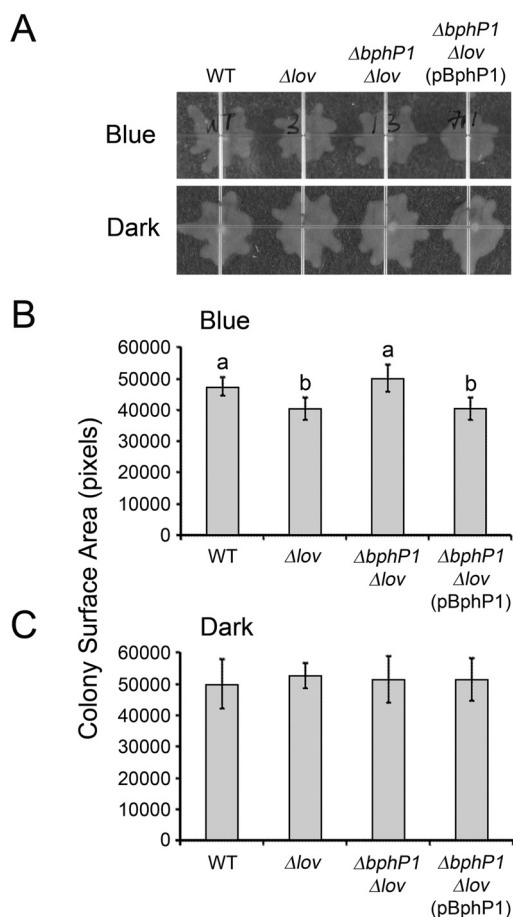


FIG 6 BphP1 suppresses swarming motility under blue light. Motility assays were performed with B728a (WT) and the Δlov , $\Delta bphP1 \Delta lov$, and $\Delta bphP1 \Delta lov$ (pBphP1) strains, and motility was photographed (A) and quantified under blue light and in the dark (B and C). Values are as described for Fig. 2.

We present the first evidence that a bacteriophytochrome responds to blue light. This finding has precedence among the plant phytochromes phyA, phyB, and phyD and the cyanobacterial phytochrome Cph2, which all can respond to blue light (17, 18, 41) but generally bind different chromophores than do the bacterio-

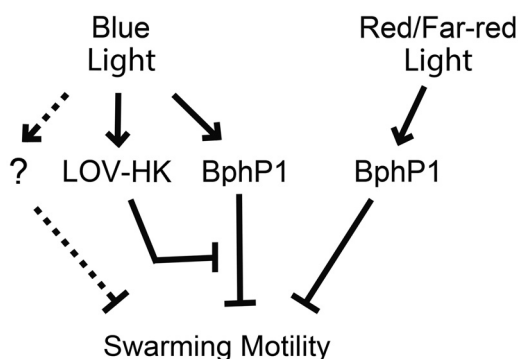


FIG 7 Model of the light-mediated LOV-HK/BphP1 signaling network in *P. syringae* B728a. BphP1 represses swarming motility in response to blue light and red/far-red light, and LOV-HK positively regulates swarming motility by suppressing BphP1-mediated blue-light signaling.

phytochromes. Our finding that a bacteriophytochrome interacts with other photosensory proteins also has precedence among plant photoreceptors. In particular, the plant phytochromes and blue-light-sensing cryptochromes have long been known to regulate the same phenotypes, such as photomorphogenesis (42), and the cryptochromes CRY1 and CRY2 physically interact with phyA and phyB, respectively (43, 44). Moreover, similar to our findings with *P. syringae* LOV-HK, cryptochromes were demonstrated to suppress phyA-, phyB-, and phyD-mediated inhibition of PIF1 (phytochrome-interacting factor 1) in *Arabidopsis* in response to blue light (41), which is similar to the suppressive effect of BphP1 on motility under blue light (see Fig. S7 in the supplemental material). Interestingly, no such interaction between plant phytochromes and phototropins, the LOV proteins of plants, has been reported, although phyA regulates the distribution of a phototropin 1 (PHOT1)-green fluorescent protein fusion protein (45).

Bacteriophytochromes photoconvert between a red-light-absorbing Pr form and a far-red-light-absorbing Pfr form. A previous study showed that both forms of BphP1 can autophosphorylate, but autophosphorylation of the Pfr form was approximately 20 times faster than that for the Pr form (8). Furthermore, once phosphorylated, both forms are capable of phosphotransfer, although the Pfr form was slightly more efficient at phosphotransfer than the Pr form (8). Our motility assays under either red or far-red light suggested that BphP1 suppressed swarming motility in response to each of these wavelengths (Fig. 4), and the histidine kinase activity was required for the biological function of BphP1 under both wavelengths. These results indicate that both the Pr and Pfr forms of BphP1 contribute to its regulation of swarming motility. phyA in plants also mediates responses to both red light and far-red light (41, 46), but whether phyA and BphP1 share a similar mechanism for these responses is not clear.

Swarming motility is a coordinated movement of a population of cells that requires, at minimum, active flagella and biosurfactant production (35). To begin to explore how light regulates swarming motility, we measured the impact of light on swimming motility, which reflects flagellar activity, and biosurfactant production, which was examined by evaluating the haloes formed by applying an atomized mineral oil spray around colonies on a solid medium, as recently described (47). The lack of significant differences between cells exposed to light and dark in their spread due to swimming motility or their production of biosurfactant (see Fig. S8 in the supplemental material) suggests that these factors are not involved in light regulation of swarming motility in this strain. The mechanism underlying this regulation remains to be determined.

We do not yet know the biological significance of light-mediated behaviors in *P. syringae*. As a foliar pathogen, the life cycle of *P. syringae* in the soil is thought to involve movement to plant seeds and eventual localization and colonization of the surface and interior of leaves. Distinct aspects of light quality could be among the many environmental cues that regulate bacterial movement, possibly helping to maximize motility on aerial plant surfaces during dark and low-light periods, when moisture levels are generally higher and therefore would support better survival. Although we identified swarming motility as a phenotypic target of LOV-HK and BphP1 in *P. syringae*, we anticipate that additional phenotypes are regulated by these proteins based on the multiple phenotypes that were found to be associated with light, as

reported for the foliar pathogen *X. axonopodis* (31) and the soil bacterial strain *R. leguminosarum* (33). Swarming motility, however, is the first phenotype shown to be regulated by both a LOV protein and a bacteriophytochrome.

The similarity shared by the LOV-HK/BphP1 network and the cryptochrome/phytochrome network of photosensing in plants (see Fig. S7 in the supplemental material) has potential evolutionary and functional implications, such as a common ancestry for these regulatory networks. LOV-HK and BphP1 are both orphan histidine kinases that do not have clear cognate response regulators. Identification of these downstream signaling components should provide insight into the biological roles of these photosensory proteins. Importantly, although the ecological role of photosensory proteins in *P. syringae* and in most other nonphotosynthetic bacteria continues to be elusive, we now have a quantitative phenotypic assay that can be used to evaluate the cellular functions of candidate interacting proteins in the LOV-HK/BphP1 regulatory networks, thus providing a practical method for identifying the downstream components of these signaling pathways.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *P. syringae* pv. *syringae* strain B728a (48) was grown in King's B medium (49) at 25°C, whereas *Escherichia coli* strain NEB10 β (New England Biolabs Inc., Massachusetts, USA), which was used for gene cloning, was grown in LB medium at 37°C. Antibiotics were added as needed at the following concentrations ($\mu\text{g ml}^{-1}$): rifampin (*rif*), 50; kanamycin (*kan*), 50; tetracycline (*tet*), 20; spectinomycin (*spc*), 20.

Generation of mutants and complementation constructs. Gene deletion mutants were constructed as described previously (50), with modifications. Specifically, we used PCR to amplify the regions flanking the target gene and also a fragment containing a *kan* cassette, followed by splice-overlap-extension PCR to generate a single fragment in which the *kan* cassette was surrounded by the flanking gene regions. We cloned the resulting fragment into the *Sma*I site of the vector pTOK2T (50) and mobilized the plasmid into B728a using the helper plasmid pRK2013 (51). Double recombinants were identified, and the *kan* cassette was evicted by introducing pFlp2 Ω , which was pFlp2Ap (52) modified to contain an *Spc*/*Sm* resistance cassette in an *Eco*RI site. pFlp2 Ω was then evicted using sucrose (10%) counterselection. After construction of the single deletion mutants for each photosensory gene, these mutants were used to generate double and triple deletion mutants. Primers for generating all of these deletion mutants are shown in Table S1 in the supplemental material.

The plasmid pLOV was constructed by cloning a fragment containing the *lov* gene under the control of its native promoter between the *Eco*RI and *Nco*I sites of the vector pME6041 (39). The plasmid pBphP1 was constructed by first cloning the promoter of the *bphO* gene, *P*_{bphO}, between the *Bgl*II and *Nco*I sites of the vector pME6031 (39) and then introducing the *bphP1* gene between the *Eco*RI and *Bam*HI sites of the pME6031-*P*_{bphO} plasmid. Site-directed mutagenesis was performed using a QuikChange II XL site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). Primers for these constructs are listed in Table S1 in the supplemental material.

Motility assays and light conditions. The motility assays were performed using 0.4% King's B agar plates that had been allowed to dry approximately 48 to 72 h on the bench, at which time their water potential was -0.7 to -1.0 MPa, as measured using a WP4 Dewpoint Potential-Meter instrument (Decagon Devices, Inc., Pullman, WA). Bacterial cells were grown in King's B broth at 25°C for 24 h in the dark. Cells were gently harvested, washed once, and resuspended with sterile Nanopure water to an optical density at 600 nm of 0.3. Two-microliter drops containing $\sim 3 \times 10^5$ cells were placed on the motility plates, with 5 drops for each of up to 6 strains placed in a randomized order in parallel rows on a single plate while working under a dim green light. Plates were incubated under var-

ious light conditions, described below, at 25°C. Motility was visible after approximately 10 h. Plates were photographed after motility was visible but before neighboring colonies contacted each other, i.e., at 10 to 15 h postinoculation. The lateral surface area of each colony was quantified using the area selection tools of Adobe Photoshop software with readings in pixels. The significance of differences among treatments was evaluated using a Student's *t* test and an analysis of variance (ANOVA) and Fisher's least-significant-difference test for multiple comparisons.

White light was provided with fluorescent bulbs (General Electric Co.), with plates placed under the bulbs at a distance such that the light intensity was $30 \mu\text{M m}^{-2} \text{s}^{-1}$. Blue (470 nm), red (680 nm), and far-red (750 nm) light was provided with light-emitting diodes from Marubeni America Corporation, with plates at a distance under the diodes such that the light intensities were 5, 10, and $0.8 \mu\text{M m}^{-2} \text{s}^{-1}$ for the blue, red, and far-red lights, respectively. The light spectra and intensity were measured with a Black-comet concave grating spectrometer (280 to 900 nm) (StellarNet Inc.) and are shown in Fig. S9 in the supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00334-13/-/DCSupplemental>.

Figure S1, TIF file, 2.7 MB.
Figure S2, TIF file, 0.9 MB.
Figure S3, TIF file, 0.6 MB.
Figure S4, TIF file, 1.8 MB.
Figure S5, TIF file, 0.8 MB.
Figure S6, TIF file, 1.3 MB.
Figure S7, TIF file, 0.2 MB.
Figure S8, TIF file, 0.8 MB.
Figure S9, TIF file, 0.6 MB.
Table S1, DOCX file, 0.1 MB.

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